

The end of the road for prostate specific antigen testing?

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Abstract

Many candidate biomarkers for diagnosis of prostate cancer have been investigated, but prostate-specific antigen (PSA) testing remains the frontline test for both mass screening and individual clinical testing. Although the PSA test is cost-effective, analytically reliable, and flexibly high throughput, it has a very weak correlation with prostate malignancy. This has resulted in over-diagnosis and over-treatment of patients leading to costly economic, social, and psychological impacts. PSA testing lacks the ability to molecularly characterize prostate diseases and define aggressiveness and lethality, which are necessary to influence choice of treatment. Therefore, newer molecular tests are beginning to replace the PSA tests. The prostate cancer antigen 3 test has shown superiority and is now widely used. The recently reported sarcosine urine test, the already delineated TMPRSS2: ETS fusion genes, the glutathione-S-transferase P1 serum marker, and enhancer of zeste homolog 2 biomarker may also help improve diagnosis and prognostication of prostate cancer. The analytical trend is toward a multiplex testing format using molecular and/or proteomic techniques that are reliable, accurate, reproducible, and ensure rapid quantitation. Therefore, validation of these newer biomarkers and their assays are necessary for both large-scale clinical trials and clinical utility.

Key words: Prostate cancer and prostate cancer antigen 3, prostate cancer antigen 3, prostate specific antigen, sarcosine

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Introduction

Recently, six new candidate biomarkers for diagnosis of prostate cancer were reported:^[1] sarcosine, uracil, kynurenine, glycerol-3-phosphate, leucine, and proline. Sarcosine, an N-methyl derivative of the amino acid, glycine, was shown to have a very strong correlation with prostate cancer progression and can be detected non-invasively using post-digital rectal examination (DRE) in urine samples of patients. Prior to the report of sarcosine test, a molecular test, the prostate cancer antigen 3 (PCA3) assay, received its European conformity mark (CE mark), and is now used in many clinical laboratories across Europe to facilitate diagnosis of prostate cancer.^[2-6] The PCA3 test, which is based on a non-coding RNA profoundly expressed in prostate tumours compared to benign cells, also uses

exfoliated urinary cells after attentive DRE.^[2] Since the clinical introduction of the PCA3 testing, several studies have compared its diagnostic and prognostic values to those of the prostate specific antigen (PSA) test for prostate cancer (PCa) management.^[4,7-15] The key comparator for all these developments remains the prostate biopsy and histology reports.^[16] The standard method of collecting prostate biopsy, the transrectal ultrasound (TRUS)-guided biopsy of 6-18 prostate cores in a patient is, without equivocation, also prone to sampling errors.^[17] In the past, the typical clinical strategy for diagnosis of prostate diseases was the triad of serum PSA test, DRE, and histology of prostate biopsy. In this strategy, the PSA assay was the most widely used but also the most controversial test. It is easily conducted in clinical laboratories as an enzyme-linked

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immunosorbent assay (ELISA) with few analytical limitations, and the PSA ELISA test can detect as low as 0.3 ng/ml of PSA in serum/plasma, but its use in prostate cancer management has many diagnostic pitfalls.

One of the problems of the PSA testing is that the biomarker itself has a weak correlation with prostate malignancy [Figure 1]; it is copiously produced by normal prostatic cells. Since its discovery in the 1970s, PSA testing has been widely used for early detection and monitoring of prostate disease. But it is never used as a one-off test. Critics of PSA testing believe that it has resulted in a huge increase in the number of prostate biopsies performed in histopathology laboratories; most of which (the PSA informed prostate biopsies) have only a 30% chance of detecting prostate cancer.^[17] Both the PSA test and the “the reference standard test” (prostate biopsy) are imperfect. Moreover, the trauma, psychological and economic impacts on patients and workload on pathology laboratories of a negative prostate biopsy result following a raised serum PSA level (PSA informed prostate biopsy) is a source of major concern. Advocates of PSA testing can point to the fact that it has increased the rate of PCa detection to about 22% over 7-year-period.^[18-20] However, increased detection rate for prostate cancer has not been convincingly linked to a reduction in mortality rate, although the recent report from the European Randomized study of Screening

for Prostate Cancer (ERSPC) showed a 20% reduction in mortality rate.^[19] However, this report was contradicted by the result of a similar study, the Prostate, Lung, Colo-rectal and Ovarian Cancer Screening trial (PLCO trial) in the USA, which reported no significant reduction in prostate cancer-specific mortality (PCSM) due to PSA screening.^[20] The controversies on PSA testing revolve around the cut-off point, diagnostic sensitivity and specificity, correlation with prostate cancer progression, pathological grade (Gleason score, GS) and tumour stage, and prediction of treatment response. In contrast, the benefits of PSA testing rely on its cost-effectiveness, analytical reliability, frontline mass screening utility, high throughput, and to a certain extent its tissue specificity. In some developing countries, with poor access to urology services, PSA testing by ELISA method may not be readily available and/or affordable. In such cases, many laboratories resort to using immunochromatographic assays (strips) which are not reliable. This review summarizes the situation with respect to PSA testing and examines a battery of laboratory tests which have been developed in attempt to improve screening, diagnosis, and monitoring of prostate cancer treatment.

The pitfalls of prostate specific antigen testing

There is a controversy on exact cut-off level for accepting normal PSA. The European Association of Urology guidelines on prostate cancer recommend <2.5-3.0 ng/ml especially for younger men (<50 years of age).^[21] In many countries including Nigeria, the 4.0 ng/ml cut-off point is commonly used, giving a diagnostic sensitivity and specificity of ~20% and 94%, respectively.^[20] A retrospective study in Nigeria reported a PSA sensitivity of 99.2% on 133 cases of hospital patients diagnosed with PCa.^[22] But the study, which had a small sample size, did not consider patients who had elevated PSA with no prostate cancer and patients with low PSA who had prostate cancer. However, in the USA, the adopted cut-off value is 2.50 ng/ml indicating a reported sensitivity and specificity of 40.5% and 81.1%, respectively.^[20] The reason for the controversy is that the diagnostic sensitivity and specificity of PSA testing vary inversely at any chosen cut-off level [Table 1].^[23] It is important to differentiate between diagnostic sensitivity and specificity of a test from its analytical sensitivity and specificity. Diagnostic sensitivity of PSA test is the ability of a raised serum/plasma PSA level above a cut-off point to correctly indicate a positive prostate cancer/disease case (confirmed by histology report). The analytical sensitivity of PSA test is the ability of the assay to quantify the lowest amount or concentration of PSA in blood sample. On the other hand, the diagnostic specificity of the PSA test is the ability of “normal” serum/plasma PSA level to correctly indicate the absence of prostate cancer/disease (also confirmed by histology report). The PSA test, determined by ELISA either manually or automated, has a good analytical sensitivity and specificity but poor diagnostic sensitivity and specificity for prostate cancer.

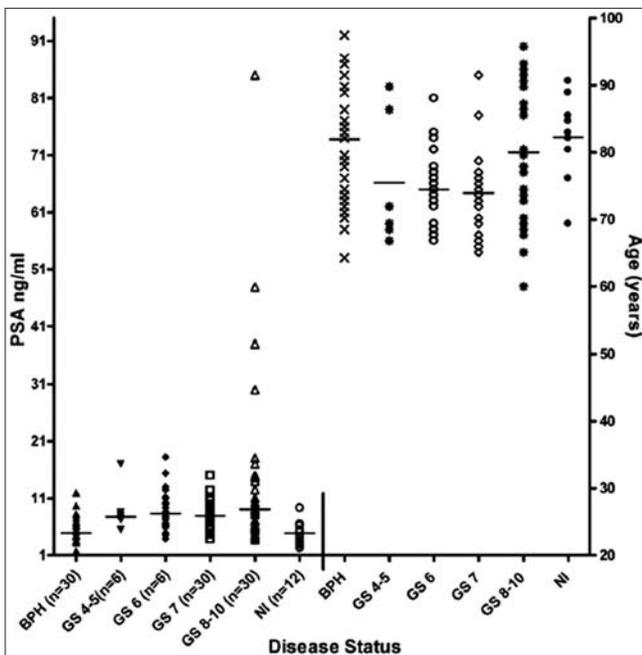


Figure 1: Pre-treatment prostate specific antigen level has no prognostic value. Figure displays the PSA level in 139 prostate disease cases.^[35] Although a small sample size, there was no significant difference in PSA level in BPH cases compared to all Gleason scores (GS) in prostate carcinomas. And within the GS, there was no significant difference in PSA level ($P > 0.05$, Kruskal–Wallis test). This group was nearly age-matched (there was no significant difference in the age group)

Table 1: Sensitivity and specificity for prostate cancer by cut points of prostate-specific antigen

Any cancer (n=1225) versus no cancer (n=1362)		
PSA, ng/ml	Sensitivity	Specificity
1.1	83.4	18.9
1.6	67.0	58.7
2.1	52.6	72.5
2.6	40.5	81.1
3.1	32.2	86.7
4.1	20.5	93.8
6.1	4.6	98.5
8.1	1.7	99.4
10.1	0.9	99.7

PSA=Prostate-specific antigen

Availability and affordability of diagnostic tests, in any country, depend largely on the healthcare management system. But these socio-economic factors do not outweigh the importance of diagnostic reliability. Even if PSA testing is made free, its diagnostic utility is still questionable. The implication of this is that the PSA as a biomarker has a weak correlation with prostate malignancy. Despite the differences in cut-off points, there are several reports of cases of prostate cancer in men with serum PSA between 2.0 and 4.0 ng/ml.^[24,25] Therefore, a low PSA level with urinary symptoms indicative of prostate problems will still require other tests to rule out prostate cancer. On the contrary, high serum levels of PSA are about 70% less likely to result in prostate cancer cases. Whether low or high, a normal or abnormal PSA co-existing with urinary symptoms associated with prostate diseases will necessarily require further/additional tests including a repeat serum PSA after an interval, DRE, the PCA3 test, and eventually a prostate biopsy for histology. To improve the diagnostic value of PSA testing, several ratiometric modifications of the test have been attempted.^[24,25] These include: Free to total PSA ratio, PSA velocity (PSAV), PSA density (PSAD), PSA doubling time, and PSA and the human glandular kallikrein 2 (hK2) product.

- The percentage free PSA (PSA ratio): This is the ratio of free-to-total PSA expressed in percentage. In routine clinical practice, a free PSA ratio of <20% in men with elevated total PSA levels (>3.0 ng/ml) is associated with a higher risk of PCa and facilitates the indication to perform a prostate biopsy.^[21] A cut-off point of 18% (free PSA ratio) was reported to increase sensitivity and specificity of PSA testing from 55% and 73% to 71% and 95%, respectively.^[13]
- PSAV: This is the rate of change of PSA over time (expressed in ng/ml/year). A PSAV >0.75 ng/ml/yr is an indicator for prostate biopsy.^[20] The PSA velocity was reported to improve sensitivity and specificity of PSA testing to >80%.^[21]
- PSAD: This is the quotient of total serum PSA by prostate volume. A value of >0.13 is considered abnormal.^[19]

- PSA doubling time: Expressed in months, this is another measure of PSA kinetics for the time it takes to reach twice the nadir concentration of PSA in blood. This is very useful in defining biochemical recurrence after primary treatment.^[21]
- PSA and hK2 product: The product of percent PSA and hK2 has been shown to enhance the diagnostic value of PSA testing especially in men with total PSA 3.0 ng/ml or greater.^[26]

Molecular and physiological forms of prostate specific antigen

PSA is a glycoprotein and its molecular mass varies depending on the analytical instrument used for its determination: Mass spectrometry, 28.5 kDa and gel electrophoresis, 30-36 kDa. The difference results from the glycosylation patterns of an N-linked oligosaccharide attached to asparagine 45.^[27] It is mostly secreted by the prostatic epithelium and the epithelial lining of the periurethral glands. However, extra-prostatic secretions of PSA from tissues such as the breast have also been reported.^[28-30] Normal prostatic epithelial cells produce more PSA glycoprotein than the malignant prostate tissues and PSA mRNA is also expressed at higher levels in benign tissue than in malignant prostatic tissues. It is believed that the PSA leaks into the blood stream as a result of deformations in the architecture of the prostate gland during trauma and/or disease.^[28] Therefore, PSA is not an ideal tumour marker because it is not produced in higher quantities by tumour cells rather there is increased leakage into blood stream as a result of tumour development.

PSA is a member of a family of serine proteases called the human tissue kallikreins. Currently, the family has 15 members that are structurally similar, hormonally-regulated, and enzymatically have either chemotrypsin- or trypsin-like activity. Their genes are tandemly localized on chromosome 19q13.4.^[29] The PSA gene is designated as KLK3 and is known to have 11 mRNA variants mainly from alternative splicing. Not all of these variants encode a mature PSA protein. The mature PSA (enzymatically active) has 237 amino acid residues and is found in both seminal fluid (SF) and serum in inactive forms. Physico-chemically, PSA exists in two states: Free and complex (PSA bound to certain plasma proteins), and in both states there can be a mixture of active and inactive forms. Inactivity of PSA results from clipping of its amino acid residues at different positions. The term total PSA refers to all immunodetectable PSA in serum/plasma including free and complex PSA.

There are five different complexes of PSA:^[28,29]

- PSA-ACT: This is PSA covalently bound to α_1 -antichymotrypsin, and is the major immunologically detectable form of PSA complex in the serum/plasma.
- PSA-MG: This is PSA covalently bound to α_2 -macroglobulin. It is not immunodetectable and thus it is often referred to as occult PSA.

- PSA-PCI: This is PSA covalently bound to protein C inhibitor; a minor component in SF, not detected in serum.
- PSA-AT: This is PSA covalently bound to α_1 -antitrypsin and its trace components are found in serum.
- PSA-IT: This is PSA covalently bound to inter-alpha trypsin inhibitor and its trace components are also found in serum.

Free PSA refers to all non-complexed PSA, which may be proteolytically active or inactive in SF, but always inactive in serum. Biologically, the PSA helps to liquefy the seminal coagulum and enhance sperm cells motility.^[19] The threshold of PSA in SF and serum/plasma varies. Large amounts of PSA (0.5-1.0 mg/ml) are present in the SF; while the serum level depends on age and prostate volume. For men up to 50 years old, the serum level is usually below 4.0 ng/ml. It can normally increase up to 6.5 ng/ml in men aged 70-79 years.^[31,32]

Prostate specific antigen and prostate cancer progression

When PSA was first identified in SF and later on in serum, it was thought to be wholly specific to the prostate and differentially expressed in normal, benign, and malignant prostate tissues.^[20,21] It therefore became widely used for screening, diagnosis, and therapeutic monitoring of prostate diseases. Its lower thresholds are within a reliable quantifiable range using the widespread ELISA assays. This was further facilitated by the commercial availability of monoclonal anti-PSA antibodies with little cross-reactivity to other members of the homologous kallikrein family.^[22] Even the detection of PSA in urine was also considered in clinical samples.^[23] However, it later began to emerge that many cases of elevated PSA had negative prostate biopsy results. In fact, it was estimated that a positive PSA result “signifies that a patient has a 25% chance for prostate cancer, which therefore causes 75% of men to have unnecessary biopsies.”^[24]

Part of the problem was that PSA testing had few randomized controlled clinical trials before it became widely entrenched in clinical use. In addition, DRE was used as the main supplement to the PSA test.^[25] Subsequent clinical trials confirmed that the PSA test was superior to DRE in detecting PCa,^[26] but the introduction, in some countries, of mass screening of older men (aged 50 years and above) for prostate cancer using the PSA test prompted a wide debate on the diagnostic value of the test. In either mass screening or individual screening where individuals decide to know their PSA levels, the debate is centered on diagnostic reliability of the PSA test. The second part of the problem is that prostate cancer is primarily a disease of old age and many people die with the disease and not from the disease. Therefore, the benefit of using PSA to detect the disease early may not necessarily translate to better survival. In fact, it could result in

over-diagnosis and over-treatment of prostate diseases. The PSA test also does little to predict the biological behaviour of prostate diseases.^[33-35]

Hence, the diagnostic, prognostic, and predictive values of the PSA test became questionable. In fact, a recent study showed a similar survival benefit for men who had “watchful waiting” in comparison with those who had radical prostatectomy.^[27] The questionable value of PSA testing may stem from the heterogeneous nature of prostate cancer and its unpredictable natural course.^[28,29] Not all histologically detectable prostate cancers are symptomatic (clinically important); and some do not even require treatment. Overall, it becomes apparent that PSA testing has a limited value in prostate cancer management; it has been enormously over-used, but its use has continued because there was no other affordable, reliable, and flexibly high throughput screening test to replace it. Lately, it has been postulated that serum PSA correlates better to progression of benign nodular hyperplasia (BPH) than PCa, merely from the fact that in most cases there are always many benign lesions around small foci of prostate cancers in a biopsy.^[30] Because of the weak correlation with prostate malignancy (and perhaps a better correlation with benign nodular hyperplasia), the PSA test is always inconclusive in both high and low levels. Consequently, the value of having a test in which a low or a high value does not necessarily affirm a positive case rather triggers a cascade of investigations that are often traumatic, expensive, and time-consuming irrespective of its outcome is the contention of many medical practitioners who fail to recommend PSA test. This does not negate the fact that there has been a significant increase in early detection of PCa through PSA testing, but the benefits of such early detections have also been controversial in the debate for a mass screening programme and/or individual screening.

The analytical strength of prostate specific antigen testing

As mentioned earlier, the analytical sensitivity and specificity of PSA testing are very reliable. There are both automated and manual systems to conduct PSA ELISA tests accurately using either spectrophotometric or immunofluorometric procedures. The test is generally cheap, flexibly high throughput, and minimally invasive (venepuncture). The PSA ELISA test is never more expensive than routine hormonal assays. Other novel test methods for PSA that could be amenable to point of care testing include electrochemical detection, optical biosensing, and mass sensitive detection.^[31] Unfortunately, the detection limits of these newer methods are not yet comparable to those of ELISAs. The availability of commercial anti-PSA antibodies and pure PSA antigens is also facilitating the progress in the development of other forms of immunometric assays. Some studies have shown that urine PSA level is not clinically useful in early detection and monitoring of prostate cancer.^[23,36-40] Other studies have also shown that

the PSA transcript numbers (copies of mRNA) in blood and urine samples are also not clinically useful for prostate cancer detection.^[32,41-45] Therefore, the ELISA remains the main technique for PSA testing. However, qualitative lateral flow assays (based on paper chromatography) have also been developed. Such assays are designed to show <4.0 ng/ml, 10 ng/ml, and >10 ng/ml points as colour bands and could prove useful though not reliable in developing countries without basic facilities for ELISA.

Mass screening for early detection of prostate cancer

The idea of using either PSA test or DRE or both to identify an early stage of prostate cancer has been a huge debate in the public health domain. An extensive review of the pros and cons of the debate is contained in a WHO paper.^[25] The reports of the long-awaited European Randomized Study of Screening for Prostate Cancer (ERSPC) and the USA Prostate, Lung, Colorectal, and Ovarian (PLCO) clinical trials have not helped matters.^[33] The ERSPC trial showed a 20% reduction in prostate cancer specific mortality (PCSM) as a result of early detection by mass screening. However, the PLCO trial showed no significant reduction in PCSM as a result of early detection by mass screening. In both trials, PSA testing was the frontline test, followed by DRE and prostate biopsy (for those with elevated PSA). The salient fact is that PSA is a weak biomarker which cannot predict the biological behaviour of prostate cancer. The problems of over-diagnosis and over-treatment come from the fact that there are currently no validated biomarkers that can differentiate aggressive from non-aggressive PCa in all cases of indolent prostate cancers detected by PSA testing.^[33-35,46-49] Although early detection of any form of disease is still a safer option to adopt whether by mass screening or individual clinical consultation, commencement and choice of treatment depend on the aggressive behaviour of the disease and the patient's choice. In the case of prostate cancer, a biomarker other than the PSA is required for mass screening to assist in identifying lethal forms of the disease.

Alternatives/supplementaries to prostate specific antigen testing

Several other prostate cancer biomarkers have been reported, which include: PCA3, sarcosine, transmembrane serine protease 2 fusion genes (TMPRSS2: ETS genes etc.), cluster of differentiation 44 (CD44), glutathione-S-transferase P1 (GSTP-1), alpha methylacyl coenzyme A racemase (AMACR), human epidermal growth factor receptor 2 (HER-2/Neu), enhancer of Zeste homolog (EZH2/histone methyl transferase); early prostate cell antigens 1 and 2, prostate-specific membrane antigen (PSMA), prostate stem cell antigen (PSCA), and ribonuclease L (RNASEL).^[33-35,46-49] The list of putative prostate biomarkers is growing rapidly, but few have been validated in larger sample studies. Reports of the evaluation of some of these biomarkers have not been consistent partly

due to lack of validation of diagnostic technologies. Many of these markers previously tested using proteomic assays can now be tested using molecular techniques including real-time PCR, which enables very sensitive, accurate, and rapid quantitation of these markers in tissue sections, exfoliated urine cells, and circulating cells in blood.^[35,50-51] Combining some of these known markers in a single test using minimally invasive sample collection methods such as urine cell sediments after attentive DRE and/or serum is a potential approach for clinical diagnosis and monitoring of prostate cancer in the post-PSA era. In addition, these putative biomarkers can also be evaluated for prognosis following primary treatment of PCa using high throughput techniques such as high-density tissue microarrays on prostate biopsies as well as real-time PCR techniques on both biopsies and urine cell sediments.^[35]

Prostate cancer antigen 3

This was first described by Bussemakers and colleagues in 1999. PCA3 gene formerly known as differential display 3 (DD3) gene is localized at chromosome 9q21 and encodes a prostate-specific RNA that is highly overexpressed in PCa tissue compared with benign prostatic tissue.^[3] The PCA3 does not encode a known protein product, suggesting that the RNA may play a regulatory role in prostate tumourigenesis. Its possible use as a urinary marker for PCa was suggested by de Kok *et al.* in 2002.^[2] The clinical assay for PCA3, a real-time PCR, was developed in the Nijmegen laboratory, the Netherlands; and results of controlled clinical trials in Canada and Austria have confirmed the potential use of the assay for PCa diagnosis.^[2] Following the acquisition of patent right from Nijmegen by Diagno cure company and later by gen-probe USA, a new quantitative molecular test based on target capture, transcription-mediated amplification, and hybridization protection, PCA3 received its CE mark in 2008 and it is now used in many clinical laboratories in Europe.^[11,36,52]

Biologically, the median upregulation of PCA3 transcript from normal to tumour tissue was reported as 34-fold, increasing to 66-fold in tumour tissues containing more than 10% cancer cells.^[37] Although the biological function of the PCA3 is unknown, its upregulation in prostate cancer tissues provided a basis for detecting the presence of the gene in tissues containing only a small number of prostate cancer cells against a background of low expression by many normal or BPH prostate cells in tissue biopsies and urine. Thus, the importance of denoting PCA3 as a ratio with PSA mRNA (a surrogate for background prostate epithelial cell nuclear material) was established.^[37] Equally important, a practical application was confirmed: The PCA3 ratio determined in voided urine, especially after light prostatic massage, or "attentive" DRE has been shown to be a sensitive and specific test for PCa.^[4,11,38,52-55] The validity of the assay depends very largely on informative specimen, that is, specimen with sufficient prostatic nuclear material measured

by PSA mRNA. The result of the assay is expressed as a score (PCA3 score) = (PCA3 mRNA/PSA mRNA) × 1000.

The diagnostic reliability of the PCA3 score has been evaluated in several studies.^[36,37] Marks *et al.* 2007 reported a sensitivity of 58% and specificity of 72% at the commonly accepted cut-off score of 35.^[36] Their report also showed an inter-run variation (precision) of about 20%. Rubrio-Briones *et al.* in 2011 reported using receiver operator curve (ROC) analysis an area under the curve (AUC) for PSA and PCA3 of 0.532 ($P = 0.417$) and 0.672 ($P < 0.0001$), respectively; sensitivities of PSA ≥ 4 ng/ml and PCA3 ≥ 35 were 87% versus 85%, with specificities of 12% versus 33%, positive predictive value (PPV) 34% versus 39% and negative predictive value (NPV) 63% versus 81%, respectively. The PCA3 score showed direct correlation with the percentage of positive biopsies ($P < 0.0001$).^[56] The PCA3 score of 35 means 35 mRNA copies of PCA3 per one copy of PSA mRNA. Fradet *et al.* (2004)^[38] reported a sensitivity of 50% and specificity of 76% at the PCA3 cut-off score of 35, with an AUC of 0.68 compared to AUC of 0.54 for PSA test. A recent study in South Africa has shown that a PCA3 score (threshold) of 60 is more discriminatory (detects more positive cases) than the conventional score of 35.^[52] Several studies have shown that the performance characteristics and diagnostic reliability of PCA3, based on sensitivity, specificity, PPV, NPV, accuracy, biopsy outcome, and AUC are superior to those of the PSA.^[3,4,13,52,54,56-58] The PCA3 test is therefore judged clinically superior to PSA test. However, there are no reported clinical trials on reduction of PCSM due to PCA3 testing.

A particularly important role of the PCA3 test appears to be in men with persistently elevated serum PSA levels (>2.50 ng/ml), but a negative initial biopsy.^[59] In such men, who constitute a large problematic group, the odds ratio for the PCA3 test to predict cancer upon re-biopsy is 3.6, compared to only 1.2 for serum PSA testing.^[37] However, at the cut-off point of 35, the PCA3 could only predict a 30% positive biopsy, increasing to 50% at PCA3 score of 100.

Currently, there is no clear-cut evidence that PCA3 score correlates strongly with GS (tumour grade) although some studies have reported a significantly lower PCA3 score in GS <7 compared to those ≥ 7 .^[60-64] Molecular grading would be invaluable in predicting the clinical outcome of intermediate GS tumors (scores: 6-7). There is also the inverse relationship between sensitivity and specificity of the PCA3 score, much similar to that of PSA. At lower cut-off points, the sensitivity increases as the specificity drops [Table 2] compared to Table 1 for PSA. However, the factors that skew PSA results such as trauma, 5 α -reductase inhibitors (finasteride and dutasteride), prostatic volume, age, inflammation, and DRE do not affect PCA3 score.^[36] PCA3 score before radical prostatectomy is known to

Table 2: Sensitivity and specificity of the prostate cancer antigen 3 assay

PCA3 score cut-off	Sensitivity (%)	Specificity (%)	Odds ratio
10	87	28	2.5
35	58	72	3.6
50	47	81	3.7

PCA3=Prostate cancer antigen 3

predict extra-capsular extension and tumour volume^[65] as well as multi-focality.^[66] The difficulty in correlating PCA3 score with prognostic and predictive markers of prostate cancer is also related to the heterogeneous natural course of the disease. Even a higher GS does not necessarily imply aggressiveness.^[33-35,49,67] There is no available information on ethnic variations of PCA3 score, if any. The test uses 20-30 ml of first catch urine after prostatic massage (2-3 massages) or attentive DRE. Without prostatic massage, the number of exfoliated cells is drastically reduced. There are possibilities that the PCA3 test can be improved by addition of other tests such as the TMPRSS2: ETS gene-fusion and KLK2 (hK2). Another important factor is that the regulation of the PCA3 gene is not clearly defined. The nucleotide sequence for the PCA3 RNA has several single nucleotide polymorphisms (SNPs), more than 100 identified SNPs (using the UCSC Genome browser). More studies are still required to define the biological role of this non-coding RNA. The PCA3 is available and affordable in most developing countries depending on their healthcare management system.

Sarcosine urine test

A report in the journal, Nature,^[11] created a huge expectation of a new test “the Sarcosine urine test.” Currently, there are no reports of successful repeat studies on the sarcosine marker in prostate cancer.^[68-71] Therefore, it is still too early to speculate on the diagnostic value of this new test. The method of sarcosine detection and quantitation would require further simplification than it was in the original report and subsequent studies.^[72-74] However, the involvement of sarcosine in the intermediary metabolic pathways that are strongly associated with prostate cancer metastasis could be of prognostic and predictive significance.^[75-80] Much work is still required to translate the sarcosine test to clinical use. The sarcosine urine test, unlike the PCA3 test, is still controversial^[69,81,82] and requires more randomized controlled clinical trials and simplified reproducible methods of analysis.

Other molecular test

Many other molecular markers [Tables 3-5] such as the ETS transcription factor fusion genes with TMPRSS2 and other 5' partners such ERG genes have been well described elsewhere^[39-41] and these markers are considered for inclusion in a multiplex testing format for a more sensitive and specific diagnosis of prostate cancer. The TMPRSS2 fusion genes

Table 3: Summary of molecular makers of prostate cancer

Gene/protein	Biological role and potential use
AKT and PTEN	Prostate-specific phosphatase and tensin homologue (PTEN) loss of function induces AKT (protein kinase) inhibiting apoptosis and may cause tubule regeneration with prostatic intraepithelial neoplasia (PIN) Use: Prognostic and therapeutic
AMACR	Alpha methylacyl coenzyme A racemase protein voided in urine. Involved in fatty acid β -oxidation. Androgen-independent function as promoter of PCa Use: Diagnostic
AR	Androgen receptor, nuclear transcription factor mediates steroid hormones and stromal cell growth. AR activation in luminal cells suppresses growth Use: Prognostic and therapeutic
Bcl-2	B-cell CLL/lymphoma 2, anti-apoptotic protein found in basal cells and stem cells. ^[2] Loss of expression linked to PIN, progression and androgen independence Use: Prognostic and therapeutic
BRCA2	Breast cancer type 2 susceptibility protein, tumor suppressor gene, predisposes to Pca, chromosome 13q. Use: Prevention
CD44	Cluster differentiation 44. Transcript variants of CD44 are associated with PCa progression and metastasis. Use: Prognostic
CgA	Chorionic gonadotropin alpha, neuroendocrine pre-hormone peptide. Unclear mechanism of action. Use: Prognostic
Cyclin D1	Role in cell cycle from G1-to S-phase Use: Prognostic
E-cadherin	Cell adhesion molecule. Downregulation/loss associated with invasion and metastasis Use: Prognostic
EGFR (Erb B1) Her-2/Neu (Erb 2)	Activation associated with proliferation, malignant transformation, relapse, progression, and androgen independence. Epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 Use: Prognostic and therapeutic
EN-2	Mouse engrailed-2 gene. Homoeobox-containing transcription factor/candidate oncogene, overexpressed in aggressive HRPC/PCa. May be positively modulated by PAX2 Use: Diagnostic and prognostic

PCa=Prostate cancer

Table 4: Summary of molecular makers of prostate cancer contd

EPCA 1 and 2	Early prostate cell antigen, nuclear matrix protein. Associated with proliferative inflammatory atrophy (PIA), PIN, and PCa. Generally, not-detected in non-cancer cases Use: Diagnostic and prognostic
ER	Oestrogen receptor located in stromal. Role unclear. Enhancer of Zeste homolog (EZH2), histone methyl transferase upregulation.
EZH2	Use: Prognostic
GSTP-1	Glutathione-S-transferase P1 protects DNA from free radicals (caretaker gene). Loss of gene expression due to hypermethylation associated with prostate cancer Use: Diagnostic
IL-6	Interleukin-6: Cytokine immunomodulator. Linked to AR cells and suppression of androgen-dependent cells. Use: Prognostic
KLK3/PSA	Encodes PSA, a kallikrein-related peptidase (serine protease subgroup) on chromosome. Use: Prognostic and therapeutic
KLK2/hK2	Encodes hK2, a kalikrein-related peptidase (serine protease subgroup) on chromosome. Serum levels 1% of PSA and undetectable in healthy males Use: Diagnostic and prognostic
MIB-1	Mindbomb homolog 1, monoclonal antibody and cell proliferation marker by Ki-67 antigen recognition. Use: Prognostic
MSMB	Microseminoprotein beta-, encodes PSP94, immunoglobulin-binding factor synthesized in prostatic epithelial cells. Use: Prognostic
NKX3.1	NK3 transcription factor related, locus 1, homoeobox tumour suppressor gene, exclusive to prostate, undergoes epigenic inactivation Use: Prognostic
NSE	Neuron-specific enolase, neuroendocrine cell product Use: Prognostic

PCa=Prostate cancer; hK2=Human glandular kallikrein 2; PSA=Prostate-specific antigen; EPCA: Early prostate cell antigen; PIN=Prostatic intraepithelial neoplasia; DNA=Deoxyribonucleic acid; AR=Androgen receptor

Table 5: Summary of molecular makers of prostate cancer contd

p27Kip1	Cell cycle inhibitor found in basal compartment. Chromosome 12p12–13.1 Use: Prognostic and therapeutic
PAP	Prostate acid phosphatase, glycoprotein more specific to prostatic tissue than PSA Use: Diagnostic and therapeutic
PCA3 (DD3)	Prostate cancer antigen 3. Chromosome 9, messenger RNA overexpressed in >95% PCa and metastases. Detected by reverse transcriptase on urine sediment. PROGENSA™ PCA3 Assay available Use: Diagnostic
PSMA	Prostate-specific membrane antigen. Androgen-independent prostatic epithelium transmembrane protein found in PCa/lymph node metastasis Use: Prognostic and therapeutic
PSCA	Prostate stem cell antigen. Membrane glycoprotein. Normal late-intermediate prostate cell marker upregulated in PCa. Use: Prognostic
p53	Tumor suppressor gene allows DNA repair/cell apoptosis in cellular stress conditions Use: Prognostic and therapeutic
RNASEL	Ribonuclease L (2',5'-oligoadenylate synthetase-dependent). Candidate tumor suppressor gene product ^[42] implicated in viral defense regulates cell proliferation and apoptosis via an interferon pathway Ch 1q23-25 Use: Diagnostic
TGF-β1	Transforming growth factor. Pleiotrophic growth factor known to promote stem cell quiescence Use: Prognostic
TMPRSS2: ETS	Transmembrane protease, serine 2 fusion gene (Ch 21), upregulates ETS target genes controlling cell proliferation, differentiation, apoptosis, and transformation Use: Prognostic and Prevention
Sarcosine	Sarcosine, an N-methyl derivative of the amino acid glycine was shown to have a very strong correlation with prostate cancer progression Use: Diagnostic
Sex hormones and binding globulin	Testosterone is essential for prostatic development and maintenance. Oestrogens are associated with low risk of PCa Use: Prognostic and prevention
u-PA/u-PAR	Urokinase plasminogen activator/cell surface receptor. Role in basement membrane/extracellular matrix degradation and metastases Use: Prognostic

PSA=Prostate-specific antigen; PCa=Prostate cancer; DD3=Differential display 3; PAP=Prostate acid phosphatase; RNA= Ribonucleic acid

occur specifically in prostate diseases, mostly in prostate cancers.^[83-85] They are also detectable in exfoliated urine cells.^[86,87] Expression of TMPRSS2 fusion genes in prostate cancer predicts unfavourable prognosis and correlates well with morphological features.^[88,89] There are now attempts to combine TMPRSS2-ETV and TMPRSS3-ERG fusion genes and PCA3 in multiplex assay formats, such as real-time PCR, using samples of exfoliated urine cells.^[90,91] These attempts reflect the heterogeneous nature of prostate cancer and the inadequacy of a single tumour marker for effective screening, grading, and monitoring of treatment.

Another consideration is the CD44 transcript variants in prostate cancer progression.^[42] Some variants of CD44 especially isoforms 3 are known to predict PSA relapse, correlate with GS and pathological grades.^[92-94] It is possible that such molecular characterization of prostate diseases may reduce the frequency of prostate biopsies and also influence the decision-making process for treatment. The GSTP-1 gene has been strongly associated with PCa progression and its testing in a panel of four genes was shown to yield a very high sensitivity and specificity (87% and 100%, respectively) for prostate cancer.^[95-99] It is another potential marker for PCa screening. The histone methyltransferase

EZH2 gene is reported to show over-expression in metastatic PCa, and this corroborates with increased amino acid metabolism and methylation associated with prostate cancer progression.^[1,75] The expression of the EZH2 gene also correlates with sarcosine expression in prostate samples.^[11] Multiplex assay formats can provide additional information on molecular characteristics of prostate diseases. Many of these tumour markers, shown in Table 3, have been known for a considerably longer time but due to lack of validated commercial assays, they have not been clinically evaluated.^[100]

Summary

In the near future, it is likely that more laboratory tests will increasingly be introduced for clinical diagnosis of prostate diseases. A typical request form for prostate profile may include PSA, hK2, and GSTP-1 blood tests; PCA3, TMPRSS2-ETS, and sarcosine urine tests. In addition, molecular tests aimed at molecular grading of prostate cancer using exfoliated urine cells from prostate massage will also help to reduce request for prostate biopsy and improve risk stratification of patients. The TMPRSS-ETV/-ERG fusion transcripts can now be tested on the same urine

samples for PCA3 tests using real-time PCR, which has a very flexible high throughput and analytical reliability. Such multiplex molecular testing may predict aggressiveness and lethality of PCa and inform better treatment choice than PSA testing. Prostate cancer will soon lend itself to better molecular characterization in exfoliated urine cells (minimally non-invasive).

There are also likely to be introduction of clinical genetic tests to assess risk of PCa development using genetic variations such as SNP, copy number variations, and hypermethylation status of some genes. This can also be done using the exfoliated urine cells from prostatic massage. Despite these developments, PSA testing is likely to remain in clinical routine screening due to availability, affordability, and familiarity but will largely be supplemented by newer molecular tests.

Conclusion

PSA testing may diminish in future to allow for a more multiplex analytes' testing for improved diagnostic sensitivity and specificity as well as better molecular characterization of biological and clinical behaviour of prostate cancer to inform a better treatment/prevention strategy. The current diagnostic strategy is unduly over-dependent on PSA testing, which has generated problematic over-diagnosis and over-treatment. Newer tests can assist to molecularly define prostate diseases, facilitating clinical discrimination of aggressive PCa from non-aggressive indolent PCa. It is therefore necessary that commercial assays such as PCA3 test be expanded into multiplex formats to include more reliable tumour markers, for example the GSTP-1 and TMPRSS2: ETS and EZH2 genes, and that such assays can serve for mass screening and individual clinical testing. The potential for combined testing of biomarkers using minimally invasive methods of sample collection such as exfoliated urine cells following DRE and/or venepuncture is to reduce unnecessary prostate biopsies and also identify clinically important PCa during screening. These benefits, which supersede those of PSA testing, will also, be required to yield a significant reduction in PCSM rate so as to justify early detection.

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